

Concentrated Ambient Air Particles Induce Vasoconstriction of Small Pulmonary Arteries in Rats

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The objective of this study was to determine whether short-term exposures to concentrated ambient particles (CAPs) alter the morphology of small pulmonary arteries in normal rats and rats with chronic bronchitis (CB). Sprague-Dawley male rats were exposed to CAPs, using the Harvard Ambient Particle Concentrator, or to particle-free air (sham) under identical conditions during 3 consecutive days (5 hr/day) in six experimental sets. CB was induced by exposure to 276 ± 9 ppm of sulfur dioxide (5 hr/day, 5 days/week, 6 weeks). Physicochemical characterization of CAPs included measurements of particle mass, size distribution, and composition. Rats were sacrificed 24 hr after the last CAPs exposure. Histologic slides were prepared from random sections of lung lobes and coded for blinded analysis. The lumen/wall area (L/W) ratio was determined morphometrically on transverse sections of small pulmonary arteries. When all animal data (normal and CB) were analyzed together, the L/W ratios decreased as concentrations of fine particle mass, silicon, lead, sulfate, elemental carbon, and organic carbon increased. In separate univariate analyses of animal data, the association for sulfate was significant only in normal rats, whereas silicon was significantly associated in both CB and normal rats. In multivariate analyses including all particle factors, the association with silicon remained significant. Our results indicate that short-term CAPs exposures (median, 182.75 µg/m³; range, 73.50–733.00 µg/m³) can induce vasoconstriction of small pulmonary arteries in normal and CB rats. This effect was correlated with specific particle components and suggests that the pulmonary vasculature might be an important target for ambient air particle toxicity. **Key words:** ambient particles, endothelial injury, pulmonary artery, rats, vasoconstriction. *Environ Health Perspect* 110:1191–1197 (2002). [Online 30 September 2002] <http://ehpnet1.niehs.nih.gov/docs/2002/110p1191-1197batalha/abstract.html>

Epidemiologic studies have observed associations between short-term increases in ambient particle concentrations and increases in respiratory and cardiovascular morbidity and mortality rates (1). Prospective cohort studies suggest that people with preexisting cardiopulmonary disease are more susceptible to the effects of urban ambient particles (2). Chronic obstructive pulmonary disease (COPD) is consistently associated with increased relative risk of death in relationship to ambient particulate air pollution (3).

Inhalation exposure of rodents to high levels of sulfur dioxide has provided an animal model similar to human COPD (4–7). Recently, this rat model of chronic bronchitis (CB) was used to study the effects of inhaled ambient particles (8,9) using the Harvard Ambient Particle Concentrator (HAPC). This device concentrates ambient outdoor particles in the fine particle range (0.1–2.5 µm) for subsequent direct delivery for animal exposure (10,11). These studies demonstrated that concentrated ambient particles (CAPs) induced changes in pulmonary breathing parameters and elicited variable degrees of pulmonary inflammation (8,9).

In animal studies, deaths associated with particle exposures have been observed in studies using a rat model of pulmonary vascular injury induced by monocrotaline (12–17),

whereas deaths were rarely observed in studies of animals with CB (18). Significant mortality was observed in the models of monocrotaline-induced pulmonary vascular injury after inhalation (13) or intratracheal instillation (12,14–16) of residual oil fly ash (ROFA) particles. Deaths associated with CAPs inhalation exposures have also been reported for the monocrotaline model (17). Additionally, spontaneously hypertensive rats have underlying pulmonary vascular disease (19) and exhibit a greater susceptibility to adverse health effects from inhaled (20) or intratracheally instilled ROFA (21).

Other studies also indicate a potentially important interaction between the inhalation of particles and pulmonary vascular injury. Short-term exposure of Sprague-Dawley rats to cigarette smoke produced pathologic features typical of pulmonary hypertension, including proliferation of cells in the endothelium and walls of the pulmonary artery (22). Moreover, in a guinea pig model of cigarette smoke-induced emphysema, the progression of histologic changes of pulmonary hypertension were greater than and dissociated from emphysema progression (23). The pulmonary vascular framework is target and host to a variety of reactions to xenobiotic exposure and injury (24). Its close anatomical and functional proximity to the

surface of the respiratory tract enhances its potential as a target for components of inhaled urban ambient particles. Our histomorphometric study focused on the small branches of pulmonary arteries adjacent to the bronchoalveolar junction of rats exposed to CAPs. Substantial concentrations of bioavailable compounds may be released from deposited ambient particles in this tissue region because this is the site of highest deposition (25–28).

The aim of this study was to assess the morphology of the pulmonary arterial vasculature in normal and CB rats and to determine whether short-term inhalation of CAPs produced any alterations measured morphometrically. Linear regression analysis was used to identify specific components of CAPs exposure associated with the measured change in vascular responses.

Materials and Methods

Experimental groups and exposure protocol. Male Sprague-Dawley rats (200–250 g) were obtained from Harlan Laboratories (Indianapolis, IN, USA) and were managed in accordance with the National Institutes of Health guidelines for care and storage of laboratory animals (29).

The model of CB was developed by exposing the rats to a target concentration of 250 ppm of sulfur dioxide (5 hr/day, 5 days/week for 6 weeks), as previously described (5,6,8). Briefly, SO₂ of known concentration was obtained from a cylinder, diluted with air, and fed into the exposure chamber at a constant rate (7.6 ft³/min). The concentration of SO₂ in the chamber was monitored continuously by an automatic flame photometric sulfur analyzer system (Meloy Labs, Inc., Springfield, VA, USA). For this purpose, a sample from the chamber was diluted 1:1,000 with room

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air and fed into the analyzer. The sulfur analysis was calibrated using an SO₂ permeation-tube calibration source (Meloy Labs). The average SO₂ concentration for all experiments was 276.4 ± 9.1 ppm. Normal control animals were maintained in the same conditions but were exposed to SO₂-free filtered air. For each experiment, CB and normal control rats were exposed to either CAPs or filtered air in sham conditions for 5 hr/day for 3 consecutive days.

Sham or CAPs exposures were administered in six different experiments, conducted in different seasons, allowing variations in ambient particle concentrations and composition. Table 1 outlines the experimental exposure design protocol, the numbers of animals per group, and the average exposure concentration. Four experimental groups were studied: *a*) 13 normal control rats exposed to filtered air (normal/sham); *b*) 15 normal control rats exposed to CAPs (normal/CAPs); *c*) 13 CB rats exposed to filtered air (CB/sham); and *d*) 15 CB rats exposed to CAPs (CB/CAPs).

Animal exposures were performed using the HAPC as previously described (8,10,11). Continuous measurements of CAPs mass concentrations were conducted using a tapered element oscillating microbalance (30). Black carbon concentrations were measured using

an aethalometer (model AE-14; Magee Scientific Inc., Berkeley, CA, USA) (31). The integrated ambient and chamber concentrations of particulate mass were determined gravimetrically. Particles were collected on preweighed 47-mm Teflon filters (P/N R2PJ047; Pall Corporation, Ann Arbor, MI) with a collection flow rate of 3 L/min. Filters were weighed using a Mettler MT-5 microbalance (Mettler Toledo, Columbus, OH) in a temperature- and humidity-controlled room. Sampling flow rate, sampling time, and filter weight were used to calculate the particle concentration (micrograms per cubic meter).

Concentrated particles were also collected on Teflon filters for measurement of sulfate, elemental carbon (EC), organic carbon (OC), and elemental analysis. Sulfate concentrations were determined using ion chromatography (32). EC and OC measurements were conducted by a thermal and optical reflectance method (33). Elemental analyses were performed by X-ray fluorescence (34). To characterize the size distribution of ambient particles, samples were collected isokinetically from the concentrator inlet using a micro-orifice uniform deposit impactor (MOUDI; MSP Corporation, Minneapolis, MN, USA) as previously described (35).

Histopathology and morphometry. The rats were euthanized using an overdose of sodium pentobarbital 24 hr after the last day (third day) of exposure. The lungs were excised and fixed by intratracheal instillation of 2.5% glutaraldehyde in 0.1 M potassium phosphate buffer at constant pressure of 20 cm H₂O. After fixation, all lung lobes (except for the cardiac lobe) were cut horizontally into uniform 2-mm sections with a guided razor blade. Each section was numbered, and one randomly selected section from each lobe was processed for histology. Tissue samples were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for analysis by light microscopy. Histologic slides were coded for blinded analysis, and the observer was unaware of the code until the analysis was completed.

For morphometric assessment, each slide was first viewed at low magnification to select arteries meeting specific criteria for measurement: *a*) each artery was adjacent to bronchoalveolar junctions (Figures 1 and 2), so that all vessels studied were in the same size range; and *b*) each artery was in true cross-section (i.e., there was a variation of < 10% between its maximum and minimum diameter) indicating that a transverse histologic section was achieved. In general, each slide contained 2 or 3 arteries meeting these criteria. Thus, we quantified 8–12 arteries per rat.

After identifying these vessels, the ratio between the lumen and wall areas (L/W) of transverse sections of pulmonary arteries at the bronchoalveolar junction was determined morphometrically using a point grid (100 points) attached to the eyepiece. Briefly, at high magnification (600–960×), the artery was placed in the center of the grid, which covered the entire

Table 1. Experimental inhalation exposure design.

Date	No. of animals	No. of animals per group				3-day mean CAPs mass concentration (μg/m ³)
		Normal/sham	Normal/CAPs	CB/sham	CB/CAPs	
March 1997	8	2	2	2	2	170.7
June 1997	8	2	2	2	2	481.0
September 1997	8	2	2	2	2	187.1
January 1998	13	3	4	3	3	126.1
February 1998	9	2	2	2	3	267.3
June 1998	10	2	3	2	3	300.7
Total	56	13	15	13	15	

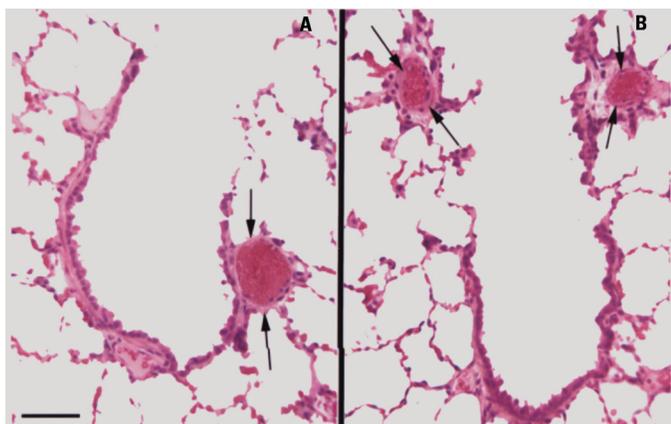


Figure 1. Morphologic appearance of lung tissue in selected areas illustrating the location of morphometric measurements, the size of the vessels studied, and the responses of normal rats to sham or CAPs exposure. (A) In normal/sham rats, the bronchoalveolar junction area has normal histology. (B) In the normal/CAPs rats, epithelial hyperplasia and arterial wall changes are visible. Note the normal pattern (arrows in A) and the thicker vessel wall (arrows in B) of small pulmonary arteries in the transverse section near the bronchoalveolar junctions. Scale bar = 50 μm for both A and B.

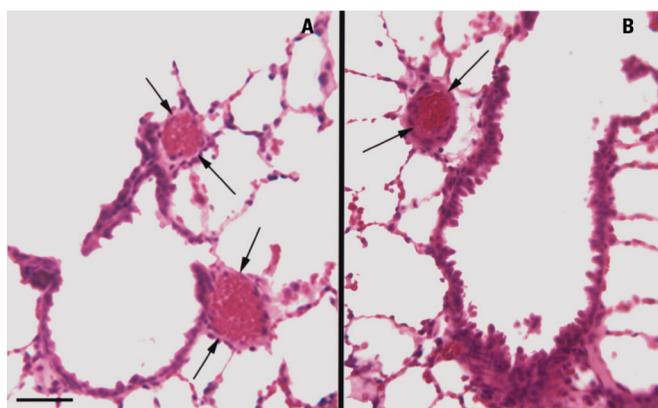


Figure 2. Morphologic appearance of lung tissue in selected areas illustrating the location of morphometric measurements, the size of the vessels studied, and the responses of CB rats to sham or CAPs exposure. (A) The bronchoalveolar junction area has airway epithelial hyperplasia and slight thickening of the vessel wall in CB/sham rats. (B) More prominent epithelial hyperplasia and vascular changes are present in CB/CAPs rats. Note an increased thickness in the vessel walls (arrows) in A and even thicker vessel walls (arrows) in the transversely sectioned small pulmonary arteries adjacent to the bronchoalveolar junctions in B. Scale bar = 50 μm for both A and B.

vessel at the magnification used. The points overlying the artery lumen and the points overlying the muscular wall were counted separately using an unbiased counting procedure as described previously (36). The concept of the L/W ratio is based on mechanisms by which vessels can narrow: *a*) muscular hypertrophy, resulting in increased thickness of the wall; *b*) constriction of the vessel, resulting in slight increases in wall thickness and diminished luminal caliber; and *c*) both of these events. Classically, measurements of pulmonary hypertension are described morphometrically in terms of measurements of medial thickness, which are used when hypertrophy is expected to dominate the pathologic process [reviewed by Wagenvoort and Wagenvoort (37)]. Because 3 days of CAPs exposure may not elicit significant hypertrophy (but may cause constriction), we employed a morphometric approach that could detect changes due to any of the mechanisms of arterial narrowing.

Statistical analysis. We calculated descriptive statistics for CAPs mass, composition, and vascular morphometric data. For the regression analyses, the L/W ratios were log-transformed to satisfy model assumptions of normality and homoscedastic variance. Three analyses of increasing sensitivity were used to detect effects of CAPs, CB status, and their interactions. First, we used analysis of variance (ANOVA) techniques to assess differences due to these three effects while treating CAPs as a binary exposure variable. Second, to assess the impact of particle mass and composition, we conducted univariate analyses regressing log L/W on differential exposure concentrations using

animals of all groups as well as stratifying by CB status. Finally, a multivariate analysis using tracer elements of previously defined pollution sources (38) as predictors was fitted to the data to confirm the univariate analyses. A principal components analysis with varimax rotation was conducted on the elemental data to confirm the ability of these tracer elements to represent the intended sources in the present experiment.

In both the ANOVA and linear regression models, experiment indicators were included as random effects in the model to account for unexplained experiment-to-experiment heterogeneity because other studies on these animals have demonstrated additional experiment-to-experiment variability unexplained by difference in exposure (39). This unexplained heterogeneity was negligible in this set of six experiments.

The particle parameters used in the univariate analyses included mass, EC, OC, sulfate, silicon, vanadium, and lead. This selection was based on the factor analysis results of the entire data set. The selected particle parameters were highly correlated with the identified factors, which usually represent a particle source(s). In previous studies, we have applied factor analysis to Boston CAPs data in an effort to identify and quantify particle sources (38). For comparability across biologic responses and elemental concentrations, results from the mixed regression models are reported as standardized regression coefficients (40). These quantities represent the change in standardized response for one unit standard deviation change in concentration. Statistical significance for all models was based on $\alpha =$

0.05. All statistical modeling was performed using SAS software (41). The mixed ANOVA and linear regression models were fitted using PROC MIXED (SAS Institute, Cary, NC, USA), whereas the factor analysis of the elemental concentrations was performed using PROC FACTOR (SAS Institute). Graphical diagnostics of model adequacy were carried out using the S-Plus statistical package (Mathsoft, Inc., Seattle, WA, USA) (42).

Results

Exposure data. Fine particle mass, sulfate, EC, OC, and elemental concentration medians, means, standard deviations, and ranges for all six experiments are presented in Table 2. Concentrated fine particle mass showed considerable variation in concentration (range = 73.5–733.0 $\mu\text{g}/\text{m}^3$). Particle composition, which was analyzed for 16 of 18 exposure days, also exhibited substantial variability. Table 3 shows the results of the factor analysis of the particle composition data. The factor loadings are reported for each of the four identified factors. The loadings, which were statistically significant, define each factor. The mean particle size (geometric standard deviation) for all experimental days was 0.27 μm (2.3). Temperature and relative humidity (mean \pm SD) in the exposure chamber for all experiments days were $28.3 \pm 1.6^\circ\text{C}$ and $45.6 \pm 6.6\%$ for sham exposures and $28.9 \pm 1.5^\circ\text{C}$ and $47.7 \pm 7.3\%$ for CAPs exposures.

Animal responses to exposure. Animals exhibited no signs of irritant inhalation or discomfort during exposure to either CAPs or filtered air. Animals mostly slept or rested quietly throughout the exposure sets. No animal died during any of the exposure days in any group.

Histopathology. Figure 1A illustrates the normal morphology of the bronchoalveolar region of the lung of normal/sham rats showing terminal airways (terminal bronchiole and alveolar duct) and cross-sectional profiles of the adjacent small pulmonary arteries typical of those that were used for analyses in these studies. In the normal/CAPs-exposed rats, there was minimal thickening of the walls of small pulmonary arteries and edema in the adventitia (Figure 1B). Morphometric measurements considered only the thickness of the muscular walls of the arteries and their lumen caliber. Perivascular edema was not included in the morphometric measurements. Minimal hyperplasia of the epithelium lining the terminal bronchiole and alveolar ducts was also present. In comparison with the normal/sham rats, the arterial walls of CB/sham (Figure 2A) and CB/CAPs (Figure 2B) rats were also thickened. Hyperplasia of the epithelium lining the terminal bronchiole and alveolar ducts was also evident and was slightly more pronounced in CAPs-exposed rats; however, the epithelial difference was not quantified morphometrically.

Table 2. Measured fine particle mass, sulfate, EC, OC, and element concentrations in the exposure chamber ($\mu\text{g}/\text{m}^3$) for all exposure days.

	Median	Mean	SD	Range
Mass	182.75	262.21	213.79	73.50–733.00
Sulfate	51.45	66.09	48.93	16.50–168.60
EC	8.05	11.45	8.27	2.40–29.71
OC	41.24	57.73	51.01	9.10–178.06
Aluminum	0.56	1.22	1.70	0.05–5.79
Silicon	3.37	4.62	4.33	0.64–13.95
Sulfur	22.01	25.61	16.59	6.26–55.58
Chlorine	0.00	0.68	1.33	1.21–4.05
Potassium	0.99	1.68	1.46	0.38–4.87
Calcium	1.19	1.82	1.73	0.26–6.00
Titanium	0.13	0.20	0.18	0.06–0.64
Vanadium	0.03	0.05	0.07	0.00–0.26
Chromium	0.01	0.01	0.01	0.00–0.03
Manganese	0.06	0.09	0.08	0.02–0.30
Iron	2.60	3.47	2.83	0.96–10.96
Nickel	0.04	0.05	0.04	0.01–0.16
Copper	0.09	0.10	0.05	0.04–0.23
Zinc	0.22	0.26	0.17	0.07–0.69
Arsenic	0.01	0.01	0.02	0.01–0.06
Selenium	0.01	0.02	0.03	0.00–0.14
Bromine	0.06	0.07	0.05	0.03–0.21
Cadmium	0.01	0.02	0.02	0.00–0.06
Barium	0.73	0.73	0.26	0.34–1.31
Lead	0.11	0.12	0.06	0.04–0.28

Data are same as in Saldiva et al. (39) and Godleski et al. (70). Reprinted from Godleski et al. (70) with permission from the British Occupational Hygiene Society.

Morphometry. Figure 3 shows box plots of the pulmonary artery L/W ratios for the four exposure groups. The medians for the four treatment groups were 2.41 for normal/sham; 1.63 for normal/CAPs; 1.66 for CB/sham; and 1.58 for CB/CAPs. The normal/sham group was significantly different ($p = 0.022$) from the other groups. When CAPs exposure was treated as a binary exposure variable, the data did not provide strong evidence of a change in the L/W ratio for either the normal ($p = 0.125$) or the CB ($p = 0.382$) rats exposed to CAPs. However, for the CAPs-exposed animals, the L/W ratio was significantly associated with particle mass and composition.

Univariate analyses were performed independently, with biologic outcome data compared with exposure data for each exposure day, followed by combinations of days. Exposure measurements for the first day had no significant associations, and all 3 days had trends similar to the data presented below. There were essentially no differences between using the second day, third day, or the mean of the second and third days (data not shown). Thus, all data presented here are from the mean concentrations for all airborne measurements from the second and third days of exposure.

The results of the individual regression analyses of L/W ratio in relationship to particle mass concentration as well as the representative particle concentrations of components vanadium, silicon, lead, sulfate, EC, and OC are shown in Table 4, which presents the regression analysis results for the total group (normal plus CB animals) and for the normal and CB groups individually. The sham group is included with zero concentration in each regression. Standardized correlation coefficient estimates, standard errors, and p -values are included. Particle mass exhibited a significant association with decreasing L/W ratio for the total group. Silicon, lead, sulfate, EC, and OC were also significantly associated with decreasing L/W in the total group. Both sulfate and silicon were significantly associated with decreasing L/W ratio in the normal group. Silicon was significantly associated in the CB

group ($p < 0.05$), as was OC ($p = 0.05$). No associations were found between vanadium concentrations and L/W for any of the three groups (total, normal, or CB). Lead was associated in the total group, but not in either individual group. The significant associations between decreasing L/W ratios and specific particle component concentrations in Table 4 indicate a dose–response relationship.

Figure 4 illustrates the dose–response relationship between the log L/W ratios and the average silicon concentration for both normal and CB rats for each experiment: log L/W decreases linearly with increases in silicon concentrations, which agrees with the results presented in Table 4. To further explore the relationship between L/W ratio and particle mass and composition, a multivariate analysis was conducted using normal and CB animals and silicon, sulfate, vanadium, and lead concentrations as particle parameters. The results of this analysis showed that only the association with silicon remained significant (coefficient = -0.146 ; $p = 0.016$).

Discussion

Our data suggest that the magnitude of the observed vasoconstrictive response to CAPs exposure is related to CAPs mass and specific particle constituent concentrations. On the basis of univariate regression analysis, we found a significant decrease of pulmonary arterial vascular L/W ratio in all rats, which was associated with silicon, sulfate, lead, EC, and OC components of CAPs. Considered separately, the CB group showed significant decreases in L/W ratio in relationship to silicon and OC concentrations. The normal rats showed significant decreases in relationship to the sulfate and silicon concentrations. Silicon showed the most significant association observed when the two groups were combined in the analysis. Furthermore, a multivariate analysis applied on the entire group showed that only the association with silicon remained significant.

In recent studies conducted in our laboratory, dogs exposed to CAPs by inhalation also exhibited significant pulmonary inflammatory

and hematologic responses to specific constituents of particles (38). Increased bronchoalveolar-lavage neutrophil percentage, total peripheral white blood cell counts, circulating neutrophils, and circulating lymphocytes were associated with increases in a factor associated with silicon. As noted above, the concentrations of silicon had the strongest associations with decreasing L/W ratio in this study reported here. It is known that the active generation of oxygen radicals on silica particle surfaces may lead to increased generation of reactive oxygen species by cells and production of proinflammatory mediators [reviewed by Devlin et al. (43)], but it is not clear whether oxygen radicals play a role in our findings. Usually, crustal elements as amorphous silicon dioxide and silicates cause no serious lung disease except at very high, occupational concentrations for a longer time than observed in CAPs exposures of our study (44,45).

The origin of silicon in CAPs is likely to be complex. Silicon concentrations were highly correlated with those of other crustal elements such as aluminum and calcium, suggesting that silicon originates from the resuspension of soil dust. Soil particles are present in coarse airborne particles (2.5–10 μm in diameter) and are usually of local origin because of their inability to travel over long distances (46). The HAPC concentrates particles $< 2.5 \mu\text{m}$ in diameter; therefore, one should expect CAPs not to contain soil particles (11). However, a fraction of soil particles are $< 2.5 \mu\text{m}$ in diameter (which is commonly called the tail of the coarse mode) and can be concentrated. Furthermore, results from our previous CAPs factor analysis/source apportionment studies have shown that silicon represents 20–30% of the mass of the silicon-related factor (38). This percentage is similar to that measured in typical particles of terrestrial origin (46).

Considering that we concentrate particles in an urban environment and that our facility is located about 75 m from a city street, it is

Table 3. Rotating factor analysis loadings of elemental composition from CAP samples (16 days of measurements).

Element	Factor 1	Factor 2	Factor 3	Factor 4
Vanadium	0.92858*	-0.08343	0.15691	0.18755
Nickel	0.92105*	-0.11394	-0.02121	-0.09134
Sulfur	0.02792	0.04290	0.15738	0.96794*
Aluminum	-0.00624	0.86625*	0.36702	0.02827
Silicon	-0.05729	0.88280*	0.06672	0.14691
Calcium	0.03489	0.84080*	0.43334	0.13444
Bromine	0.10149	0.20709	0.92071*	0.19574
OC	0.78321*	0.23076	0.46400	0.32122
EC	0.63524	0.25281	0.46245	0.49525
Lead	0.21533	0.42040	0.77201*	0.36184

Data are the same as in Godleski et al. (70), reprinted with permission from the British Occupational Hygiene Society.

*Significant associations defining the factor.

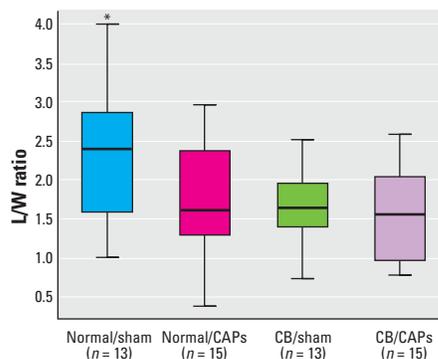


Figure 3. Box plot representing the distribution of the pulmonary artery L/W ratio measurements for the four exposure groups.

*The normal/sham group is significantly different from the other three groups ($p = 0.022$).

possible that CAPs include road dust particles in addition to others that are more directly associated with emission from combustion sources. Although nontoxic silicon and aluminum oxides represent a large fraction of the urban road dust, these particles have been continuously enriched with combustion-derived material, organic semivolatile compounds, and acidic inorganic gases (47). In addition, debris from cars, such as tire- and brake-derived particles, can constitute a large fraction of road dust; pollen and other bioaerosols can also be abundant in road dust (48). Therefore, although silicon concentrations as representative of pure soil particles are not expected to induce adverse effects, it is conceivable that silicon is a surrogate of road dust that encompasses a large number of components that may be responsible for the observed effects reported in this present study as well as our previous investigations. In future studies, we plan to use single particle analysis to investigate the size and composition of the silicon-related particles. Our results support an association between L/W ratio and OC, which is weakly correlated with silicon, as shown in Table 3. Road dust particles, rich in OC, may induce the observed effects. Therefore, a better characterization of particle physicochemical properties using single particle analysis will be very important for our studies.

In the dog study (38), increased circulating neutrophils and increased bronchoalveolar lavage macrophages were also associated with the vanadium and nickel factor, which was not associated with the vascular L/W changes in this rat study. Significant decreases in red blood cell counts and hemoglobin levels of dogs were correlated with the sulfur factor, which also had a significant effect on decreasing vascular L/W ratio in our study. Both the dog and rat studies show specific components of ambient air particles to be more important than mass in the development of biologic responses.

In the same rats used for the analyses reported here, studies focusing on lung inflammation showed a significant increase in bronchoalveolar lavage neutrophils and neutrophils in the lung parenchyma with CAPs exposure (39). CAPs compositional

analyses demonstrated a significant and dose-dependent association in the last 2 days of exposure between bronchoalveolar lavage neutrophils and tissue neutrophil density with vanadium, bromine, and lead concentrations. Bronchoalveolar lavage neutrophils and protein concentrations were also significantly associated with increases in concentrations of lead, sulfate, silicon, OC, and EC, which all were associated with decreasing L/W ratio in our study.

A noteworthy finding of these studies is the observed association of biologic responses and individual particle composition, suggesting that the bioavailability and activity of some ambient particles components in specific sites may play a fundamental role in the pathogenic events. Despite the observed correlations, it is not possible to identify whether the observed changes are due to isolated or synergistic effects among the particle components. Urban aerosols represent a complex mixture of anthropogenic and naturally occurring airborne particles either emitted directly by pollution sources, generated by reactions that occur in the atmosphere, or produced by mechanical resuspension (46). We used urban Boston outdoor air typically composed of particles generated by vehicle exhaust, power plant emissions, home heating, and transported aerosols (49,50). There was substantial variability in exposure data in these studies. Variation in particle mass and elemental concentrations is related to emissions, weather, air mass trajectories, wind directions, and season. Nevertheless, the variability in both exposure data and biologic response provided the potential to better characterize the associations.

Our results indicate that short-term inhalation exposures to CAPs can promote vasoconstriction of small pulmonary arteries in normal and CB rats, suggesting that the pulmonary vasculature may be a target for the effects of ambient particles. These morphologic findings are in agreement with other morphologic studies of small pulmonary vessel injury in dogs from areas of high ambient pollution in Mexico City (51). Furthermore, because the animals of the study reported here were sacrificed using an overdose of pentobarbital anesthesia, it is possible that our morphometric studies underestimate the magnitude of the

changes because it is known that pentobarbital used as an anesthetic can attenuate vasoconstriction (52,53). In addition, a number of recent studies of pulmonary endothelium-derived mediators responding to ambient particle exposures have been reported (54).

The most important regulator of pulmonary vascular tone and blood flow is the balance of vasoconstrictive and vasodilative mediators. The endothelium-derived constricting factors include a family of three endothelin isoforms (ET-1, ET-2, and ET-3) (55). These effects are counterbalanced by the pulmonary vascular endothelium's ability to release endothelium-derived relaxing factors (56), which includes nitric oxide (NO) (57,58) and prostacyclin (59). Increased plasma levels of ET-1 were observed in rats after inhalation of urban particles (60). Furthermore, human volunteers exposed to urban particulate matter < 2.5 μm in diameter (PM_{2.5}) by inhalation had increased ET-1 and ET-3 plasma concentrations (61). Cigarette smoke also increases plasma ET-1 plasma levels (62). Whether some bioavailable components of ambient particles induce lung vascular endothelial cells to alter the release, turnover, or receptor activity (regulation) of vascular mediators is unclear at this time. Pulmonary endothelial dysfunction, represented by an increase in vasoconstrictive activity locally through an autocrine/paracrine effect on the pulmonary vasculature, could explain the observed vasoconstriction in our study. Perhaps an endocrine effect at the level of the coronary vasculature could lead to a vasoconstrictive effect with enhanced ischemia and predisposition to arrhythmias (63–66). This attractive hypothesis could suggest a plausible pathogenic pathway to the increased morbidity and mortality associated with pre-existing cardiovascular and respiratory diseases observed in epidemiologic studies.

The pathologic mechanism by which particles result in pulmonary vasoconstriction may be similar to that observed for other pulmonary vasoconstrictors. For instance, the

Table 4. Standardized correlation coefficient estimates (SEs) and *p*-values from univariate linear regression model analyses of log vascular L/W ratio and particle exposure parameters for the total group (normal plus CB animals) and for normal and CB groups.

	CAPs mass	Vanadium	Silicon	Lead	Sulfate	EC	OC
Total	−0.345 (0.140) <i>p</i> = 0.0170*	−0.182 (0.128) <i>p</i> = 0.1601	−0.490 (0.136) <i>p</i> = 0.0007*	−0.297 (0.134) <i>p</i> = 0.0310*	−0.388 (0.136) <i>p</i> = 0.0062*	−0.360 (0.135) <i>p</i> = 0.0104*	−0.328 (0.135) <i>p</i> = 0.0189*
Normal	−0.281 (0.158) <i>p</i> = 0.1019	−0.155 (0.154) <i>p</i> = 0.3189	−0.441 (0.141) <i>p</i> = 0.0031*	−0.203 (0.154) <i>p</i> = 0.1937	−0.313 (0.147) <i>p</i> = 0.0308	−0.277 (0.150) <i>p</i> = 0.0723	−0.210 (0.150) <i>p</i> = 0.1689
CB	−0.267 (0.149) <i>p</i> = 0.0793	−0.147 (0.145) <i>p</i> = 0.3169	−0.291 (0.144) <i>p</i> = 0.0494*	−0.267 (0.149) <i>p</i> = 0.0805	−0.281 (0.150) <i>p</i> = 0.0670	−0.283 (0.149) <i>p</i> = 0.0638	−0.289 (0.145) <i>p</i> = 0.0520*

*Significant association.

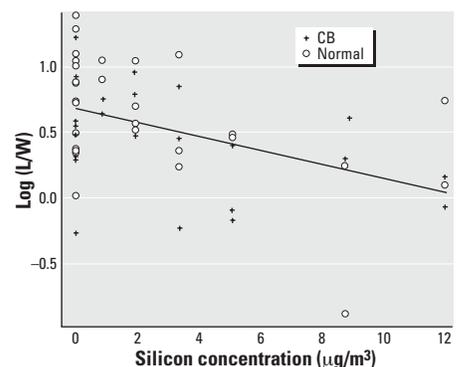


Figure 4. Dose–response relationship between log L/W ratios and the 2-day mean silicon concentration for both normal and CB rats.

monocrotaline-induced model of pulmonary vascular injury in rats is characterized by progressive pulmonary hypertension and histopathologic lung alterations, including progressive capillary endothelial cell damage, medial thickening of smooth muscle layer of small- and medium-sized pulmonary arteries, and fragmentation of elastic laminae in main pulmonary arteries. Our study shows thickening of pulmonary arteries at the light microscopic level. In the animal model of monocrotaline-induced pulmonary hypertension (67,68), endothelial ET-1 production is elevated and NO activity is decreased. The fact that deaths occur in animals of this model when exposed to particles raises the possibility that the same mechanism involved in response to monocrotaline is operative in response to particle inhalation. In addition, in the animal model of CB, the observed vasoconstriction could be explained also by a predominant action of endothelium-derived constricting factors. Decreased NO activity and increased plasma levels of ET-1 in response to hypoxemia have been described and also could explain the pulmonary arterial vasoconstriction and pulmonary hypertension observed in COPD (56,69).

Thus, vasoconstriction of pulmonary vessels associated with CAPs exposures may be the result of an effect at the level of the pulmonary vascular endothelial cells with an abnormal balance between releasing of endothelium-derived constricting factors and endothelium-derived relaxing factors. We speculate that the resultant pulmonary endothelial dysfunction with predominant release of mediators that constrict vessels could lead to a dominant vasoconstrictive status in the lungs and possibly in the heart. These same mediators could potentiate altered hemostasis with increased platelet activation and enhanced blood coagulability and thrombogenesis. In animal studies, significant mortality associated with ambient or surrogate particle inhalation or intratracheal instillation has been observed only in models where there was a preexisting endothelial injury. Human epidemiologic studies show that the increased risk of death is greater in people with COPD (3), a disease process that includes some pulmonary endothelial dysfunction (69). Further studies of CAPs on the pulmonary arterial vasculature are needed to establish the full extent and the chronicity of these demonstrated effects.

REFERENCES AND NOTES

- Pope CA III. Epidemiology of fine particulate air pollution and human health: biologic mechanisms and who's at risk? *Environ Health Perspect* 108(suppl 4):713–723 (2000).
- Dockery DW, Pope CA, Xu X, Spengler JD, Ware JH, Fay ME, Ferris BG, Speizer FE. An association between air pollution and mortality in six U.S. cities. *N Engl J Med* 329:1753–1759 (1993).
- Schwartz J. What are people dying of on high air pollution days? *Environ Res* 64:26–35 (1994).
- Lamb D, Reid L. Mitotic rates, goblet cell increase and histochemical changes in mucus in rat bronchial epithelium during exposure to sulphur dioxide. *J Pathol Bacteriol* 96:97–111 (1968).
- Farone A, Huang S, Paulauskis JD, Kobzik L. Airway neutrophilia and chemokine mRNA expression in SO₂-induced bronchitis. *Am J Respir Cell Mol Biol* 12:345–350 (1995).
- Shore S, Kobzik L, Long N, Skornik W, Van Staden C, Boulet L, Rodger IW, Pon DJ. Increased airway responsiveness to inhaled methacholin in a rat model of chronic bronchitis. *Am J Respir Crit Care Med* 151:1931–1938 (1995).
- Sweeney TD, Skornik WA, Brain JD, Hatch V, Godleski JJ. Chronic bronchitis alters the pattern of aerosol deposition in the lung. *Am J Respir Crit Care Med* 151(2 pt 1): 482–488 (1995).
- Clarke RW, Catalano PJ, Koutrakis P, Krishna Murthy GG, Sioutas C, Paulaskis J, Coull BA, Ferguson S, Godleski JJ. Urban air particulate inhalation alters pulmonary function and induces pulmonary inflammation in a rodent model of chronic bronchitis. *Inhal Toxicol* 11:637–656 (1999).
- Kodavanti UP, Mebane R, Ledbetter AD, Krantz T, McGee J, Jackson MC, Walsh L, Hilliard H, Chen BY, Richards JR, et al. Variable pulmonary responses from exposure to concentrated ambient air particles in a rat model of bronchitis. *Toxicol Sci* 54:441–451 (2000).
- Sioutas C, Koutrakis P, Burton RM. A technique to expose animals to concentrated fine ambient aerosols. *Environ Health Perspect* 103:172–177 (1995).
- Sioutas C, Koutrakis P, Godleski JJ, Ferguson ST, Kim CS, Burton RM. Fine particle concentrators for inhalation exposures—effect of particle size and composition. *J Aerosol Sci* 28:1057–1071 (1997).
- Costa DL, Lehmann JR, Frazier LT, Doerfler D, Ghio A. Pulmonary hypertension: a possible risk factor in particulate toxicity [Abstract]. *Am J Resp Crit Care Med* 149(A):840 (1994).
- Killingsworth CR, Alessandrini F, Krishna Murthy GG, Catalano PJ, Paulauskis JD, Godleski JJ. Inflammation, chemokine expression, and death in monocrotaline-treated rats following fuel oil fly ash inhalation. *Inhal Toxicol* 9:541–565 (1997).
- Kodavanti UP, Jacson M, Gardner SY, Watkinson WP, Campen MJ, Richards J, Costa DL. Particle-induced lung injury in hypertensive rats [Abstract]. *Am J Resp Crit Care Med* 155:A247 (1997).
- Watkinson W, Campen MJ, Costa DL. Cardiac arrhythmias induction after exposure to residual oil fly ash particles in a rodent model of pulmonary hypertension. *Toxicol Sci* 41(2):209–216 (1998).
- Kodavanti UP, Jackson MC, Ledbetter AD, Richards JR. Lung injury from intratracheal and inhalation exposures to residual oil fly ash in a rat model of monocrotaline-induced pulmonary hypertension. *J Toxicol Environ Health* 57(8):543–563 (1999).
- Lovett E, Clarke RW, Verrier RL, Koutrakis P, Lawrence J, Antonini JM, Godleski JJ. Rat cardiovascular dysfunction prior to death during exposure to concentrated ambient air particles [Abstract]. *Toxicologist* 48(1S):297 (1999).
- Godleski JJ, Sioutas C, Katler M, Koutrakis P. Death from inhalation of concentrated ambient air particles in animal models of pulmonary disease [Abstract]. *Am J Respir Crit Care Med* 153:A15 (1996).
- Aharinejad S, Schraufnagel DE, Böck P, MacKay CA, Larson EK, Miksovsky A, Marks SC Jr. Spontaneously hypertensive rats develop pulmonary hypertension and hypertrophy of pulmonary venous sphincters. *Am J Pathol* 148:281–290 (1996).
- Kodavanti UP, Schladweiler MCJ, Ledbetter AD, Watkinson WP, Campen MJ, Winset DW, Richards JR, Crissman KM, Hatch GE, Costa DL. The spontaneously hypertensive rat as a model of human cardiovascular disease: evidence of exacerbated cardiopulmonary injury and oxidative stress from inhaled emission particulate matter. *Toxicol Appl Pharmacol* 164:250–263 (2000).
- Kodavanti UP, Schladweiler MCJ, Richards JR, Costa DL. Acute lung injury from intratracheal exposure to fugitive residual oil fly ash and its constituent metals in normo- and spontaneously hypertensive rats. *Inhal Toxicol* 13:37–54 (2001).
- Sekhon H, Wright JL, Churg A. Cigarette smoke causes rapid cell proliferation in small airways and associated pulmonary arteries. *Am J Physiol Lung Cell Mol Physiol* 267(1):L557–L563 (1994).
- Wright J, Churg A. Effect of long-term cigarette smoke exposure on pulmonary vascular structure and function in the guinea pig. *Exp Lung Res* 17:997–1009 (1991).
- Boor P, Gottlieb AI, Joseph EC, Kerns WD, Roth RA, Tomaszewski KE. Chemical-induced vasculature injury. *Contemp Issues Toxicol* 132:177–195 (1995).
- Brody AR, Roe MW. Deposition pattern of inorganic particles at the alveolar level in the lungs of rats and mice. *Am Rev Respir Dis* 128(4):724–729 (1983).
- Warheit DB, Hartsky MA. Assessments of pulmonary macrophage clearance responses to inhaled particulates. *Scanning Microsc* 2(2):1069–1078 (1988).
- Zeltner TB, Sweeney TD, Skornik WA, Feldman HA, Brain JD. Retention and clearance of 0.9-micron particles inhaled by hamsters during rest or exercise. *J Appl Physiol* 70(3):1137–1145 (1991).
- Churg A, Brauer M. Ambient atmospheric particles in the airways of human lungs. *Ultrastruct Pathol* 24(6):353–361 (2000).
- ARENA, OLAW. Institutional Animal Care and Use Committee Guidebook. 2nd ed. Bethesda, MD:Office of Laboratory Animal Welfare, National Institutes of Health, and Applied Research Ethics National Association, 2002.
- Pataschnick H, Rupperecht E. Continuous PM-10 measurements using the tapered element oscillating microbalance. *J Air Waste Manag Assoc* 41:1079–1083 (1991).
- Hansen A, Rosen H, Novakov T. The aethalometer—an instrument for real-time measurement of optical absorption by aerosol particles. *Sci Total Environ* 36:191–196 (1984).
- Koutrakis P, Wolfson JM, Spengler JD. An improved method for measuring aerosol acidity: results from a nine-month study in St Louis MO, and Kingston TN. *Atmos Environ* 22:157–163 (1988).
- Chow J, Watson JG, Prichett LC, Pierson WR, Frazier CA, Purcell RG. The DRI thermal/optical reflectance carbon analysis system: description, evaluation and applications in US air quality studies. *Atmos Environ* 27A:1185–1201 (1993).
- Jaklevic J, French WR, Clarkson TW, Greenwood MR. X-ray fluorescence analysis applied to small samples. *Adv X-Ray A21:171–185 (1977)*.
- Marple V, Rubow KL, Behm SM. A micro orifice uniform deposit impactor (MOUDI): description, calibration, and use. *Aerosol Sci Technol* 14:434–446 (1991).
- Warth MPN, Maldonado EAL, Fernandez SM, Leme AS, Perini A, Saldiva PHN, Martins MA. Neurokinin depletion attenuated mechanical and morphological pulmonary changes induced by antigen challenge in sensitized guinea pigs. *Am J Physiol Lung Cell Mol Physiol* 268:L781–L788 (1995).
- Wagenvoort CA, Wagenvoort N. Normal anatomy and responses to disease. In: *Pulmonary Vascular Diseases*. (Moser KM, ed). New York: Marcel Dekker, 1979;1–109.
- Clarke RW, Coull BA, Reinisch U, Catalano PJ, Killingsworth CR, Koutrakis P, Kavouros I, Krishna Murthy GG, Lawrence J, Lovett E, et al. Inhaled concentrated ambient particles are associated with hematologic and bronchoalveolar lavage changes in canines. *Environ Health Perspect* 108:1179–1187 (2000).
- Saldiva PHN, Clarke RW, Coull BA, Stearns RC, Lawrence J, Krishna Murthy GG, Diaz E, Koutrakis P, Suh H, Tsuda A, et al. Acute pulmonary inflammation induced by concentrated ambient air particles is related to particle composition. *Am J Resp Crit Care Med* 165:1610–1617 (2002).
- Zar J. *Biostatistical Analysis*. Upper Saddle River, NJ: Prentice-Hall, 1996.
- SAS Institute. *SAS/STAT User's Guide*, Version 8. Cary, NC:SAS Institute, Inc., 1999.
- Venables W, Ripley BD. *Modern Applied Statistics with S-Plus*. New York:Springer, 1994.
- Devlin, RB, Ghio AJ, Costa DL. Molecular and cellular responses of the lung to inhaled particles: responses of inflammatory cells. In: *Particle-Lung Interactions* (Gehr P, Heyder J, eds). New York:Marcel Dekker Inc, 2000;437–472.
- McLaughlin J, Chow WH, Levy LS. Amorphous silica: a review of health effects from inhalation exposure with particular reference to cancer. *J Toxicol Environ Health* 50(6):553–566 (1997).
- Godleski, J. The pneumoconioses: silicosis and silicatosis. In: *Pathology of Pulmonary Disease* (Saldana M, ed). Philadelphia:J.B. Lippincott Co., 1994;387–393.
- Hinds W, ed. *Atmospheric Aerosols in Aerosol Technology*. New York:John Wiley & Sons, 1999.

47. Yang HH, Chiang CF, Hee WJ, Hwang KP, Wu EMY. Size distribution and dry deposition of road dust PAHs. *Environ Int* 25(5):585–597 (1997).
48. Miguel AG, Cass GR, Glovsky MM, Weiss J. Allergens in paved road dust and airborne particles. *Environ Sci Toxicol* 33(23):4159–4168. (1999).
49. Spengler J, Thurston G. Mass and elemental composition of fine and coarse particles in six U.S. cities. *J Air Pollut Control Assoc* 3:1162–1171 (1983).
50. Oh JA, Suh HH, Lawrence JE, Allen GA, Koutrakis P. Characterization of particulate mass concentrations in South Boston, MA. In: *Proceedings of AWMA/EPA Symposium on Measurement of Toxic and Related Air Pollutants*, Research Triangle Park, NC, 29 April–1 May 1997. AWMA publication VIP-74. Pittsburgh, PA:Air and Waste Management Association, 1997;397–407.
51. Calderon-Garciduenas L, Mora-Tiscareno A, Fordham LA, Chung CJ, Garcia R, Osnaya N, Hernandez J, Acuna H, Gambling TM, Villarreal-Calderon A, et al. Canines as sentinel species for assessing chronic exposures to air pollutants: part 1. Respiratory pathology. *Toxicol Sci* 61(2):342–355 (2001).
52. Wetzel RC, Martin LD. Pentobarbital attenuates pulmonary vasoconstriction in isolated sheep lungs. *Am J Physiol* 257(3 Pt 2):H898–H903 (1989).
53. Nyhan DP, Goll HM, Chen BB, Fehr DM, Clougherty PW, Murray PA. Pentobarbital anesthesia alters pulmonary vascular response to neural antagonists. *Am J Physiol* 256(5 Pt 2):H1384–H1392 (1989).
54. Vincent R, Kumarathasan P, Goegan P, Bjarnason SG, Guenette J, Berube D, Adamson IY, Desjardins S, Burnett RT, Miller FJ, et al. Inhalation toxicology of urban ambient particulate matter: acute cardiovascular effects in rats. *Res Rep Health Eff Inst* (104):5–54 (2001). Available: <http://www.healtheffects.org/Pubs/Vincent.pdf> [cited April 2002].
55. Michael J, Markewitz BA. Endothelins and the lung. *Am J Respir Crit Care Med* 154:555–581 (1996).
56. Chen Y, Oparil S. Endothelial dysfunction in the pulmonary vascular bed. *Am J Med Sci* 320(4):223–232 (2000).
57. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 43:109–142 (1991).
58. Adnot S, Raffestin B, Eddahibi S. NO in the lung. *Respir Physiol* 101:109–120 (1995).
59. Kerins DM, Murray R, FitzGerald GA. Prostacyclin and prostaglandin E1: molecular mechanisms and therapeutic utility. *Prog Hemostasis Thromb* 265:C1620–C1629 (1991).
60. Bouthillier L, Vincent R, Goegan P, Adamson IY, Bjarnason S, Stewart M, Guenette J, Potvin M, Kumarathasan P. Acute effects of inhaled urban particles and ozone: lung morphology, macrophage activity, and plasma endothelin-1. *Am J Pathol* 153:1873–1884 (1998).
61. Vincent R, Kumarathasan P, Mukherjee B, Gravel C, Bjarnason S, Urch B, Speck M, Brook J, Tarlo S, Zimmerman B, et al. Exposure to urban particles (PM_{2.5}) causes elevations of the plasma vasoactive endothelin ET-1 and ET-3 in humans [Abstract]. *Am J Resp Crit Care Med* 163:A313 (2001).
62. Haak T, Jungmann E, Raab C, Usadel KH. Elevated endothelin-1 levels after cigarette smoking. *Metabolism* 43(3):267–269 (1994).
63. Godleski JJ, Verrier RL, Koutrakis P, Catalano P. Mechanisms of morbidity and mortality from exposure to ambient air particles. *Res Rep Health Eff Inst* (91):5–85 (2000). Available: <http://www.healtheffects.org/Pubs/Godleski.pdf> [cited April 2002].
64. Peters A, Liu E, Verrier RL, Schwartz J, Gold DR, Mittleman M, Baliff J, Oh A, Allen G, Monahan K, et al. Air pollution and incidence of cardiac arrhythmia. *Epidemiology* 11:11–17 (2000).
65. Wellenius GA, Saldiva PHN, Batalha JRF, Krishna Murthy GG, Coull BA, Verrier RL, Godleski JJ. Exposure to residual oil fly ash (ROFA) particles in a rat model of myocardial infarction. *Toxicol Sci* 66:327–335 (2002).
66. Santos UP, Lin CA, Pereira LAA, Vieira T, Braga ALF, Saldiva PHN, Terra Filho M. Association between air pollution and cardiac arrhythmia in Sao Paulo, Brazil [Abstract]. *Am J Respir Crit Care Med* 163:A236 (2001).
67. Mathew R, Zeballos GA, Tun H, Gewitz MH. Role of nitric oxide and endothelin-1 in monocrotaline-induced pulmonary hypertension in rats. *Cardiovasc Res* 30:739–746 (1995).
68. Frasch H, Marshall C, Marshall BE. Endothelin-1 is elevated in monocrotaline pulmonary hypertension. *Am J Physiol* 276:L304–L310 (1999).
69. Faller M, Kessler R, Sapin A, Chaouat A, Ehrhart M, Ducoloné A, Weitzenblum E. Regulation of endothelin-1 at rest and during a short steady-state exercise in 21 COPD patients. *Pulm Pharmacol Ther* 11:151–157 (1998).
70. Godleski JJ, Clarke RW, Coull BA, Saldiva PHN, Jiang NF, Lawrence J, Koutrakis P. Composition of inhaled urban air particles determines acute pulmonary responses. *Ann Occup Hyg* 46(suppl 1):419–424. (2002).